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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/417,386	10/13/1999	JONATHAN M. ROTHBERG	15966-539	7371

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EXAMINER	
TAYLOR, JANELL E	
ART UNIT	PAPER NUMBER
1634	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/417,386	Applicant(s) ROTHBERG ET AL.	
	Examiner Janell Cleveland Taylor	Art Unit 1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 6 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *29 July 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3-7,9-19 and 27-32* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3-7,9-19 and 27-32* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☒ Other: *Detailed Action* .

DETAILED ACTION

Request for Continued Examination

1. The request filed on August 14, 2002 for a Request for Continued Examination (RCE) under 37 CFR 1.53(d) is acceptable and a CPA has been established. An action on the RCE follows.

2. The following action is NON-FINAL. Any rejection not reiterated is withdrawn. A Response to Arguments section follows.

Claim Rejections - 35 USC § 102

1. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

2. Claims 1, 31, 19, and 27 are rejected under 35 U.S.C. 102(e) as being anticipated by Austin et al. (USPN 6,132,965).

Austin et al. teaches "RNA prepared by conventional methods from a first cell population and RNA from a second cell population are separately reverse-transcribed and second-strand synthesized to form two pools of double-stranded cDNA (*providing a population of cDNA molecules derived from a population of RNA molecules*), a tester pool comprising sequences of the mRNA species desired to be enriched for, and a driver pool comprising the sequences desired to be subtracted from the tester pool. The two pools may be fragmented by endonuclease digestion (restriction endonuclease or

non-specific endonuclease) if desired to enhance hybridization efficiency. (*partitioning said population into one or more subpopulations of nucleic acids, wherein said partitioning comprises digesting the cDNA molecules with one or more restriction enzymes*). The driver pool and tester pool are denatured and mixed together in a reaction mixture under hybridization conditions and incubated for a suitable hybridization period. The reaction mixture is contacted with a ligand which binds the recoverable label on the driver cDNA and which can be readily recovered from the reaction mixture (e.g., avidin attached to magnetic beads), such that a substantial fraction of the driver cDNA and any tester cDNA hybridized thereto is selectively removed from the reaction mixture. (*determining whether the first nucleic acid sequence is identical to a reference nucleic acid sequence, whereby the first nucleic acid is novel if the first nucleic acid sequence is not identical to the reference nucleic acid sequence*). The enriched (subtracted) tester cDNA pool may be subjected to one or more additional rounds of subtractive hybridization with a pool of labeled driver cDNA..." (Col. 22, lines 40-70). "cDNA species remaining in the tester pool can be cloned, amplified, and/or sequenced for unambiguous identification, such as in the form of an EST sequence." (Col. 23) (*sequencing at least one nucleic acid sequence in the subpopulation to provide a first nucleic acid sequence*).

Therefore, Austin teaches all of the limitations of claims 1, 19, and 27.

Claim Rejections - 35 USC § 103

3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

4. Claims 3-7, 18, and 20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Austin et al.

As disclosed above, Austin et al. teaches "RNA prepared by conventional methods from a first cell population and RNA from a second cell population are separately reverse-transcribed and second-strand synthesized to form two pools of double-stranded cDNA a tester pool comprising sequences of the mRNA species desired to be enriched for, and a driver pool comprising the sequences desired to be subtracted from the tester pool. The two pools may be fragmented by endonuclease digestion (restriction endonuclease or non-specific endonuclease) if desired to enhance hybridization efficiency. The driver pool and tester pool are denatured and mixed together in a reaction mixture under hybridization conditions and incubated for a suitable hybridization period. The reaction mixture is contacted with a ligand which binds the recoverable label on the driver cDNA and which can be readily recovered from the reaction mixture (e.g., avidin attached to magnetic beads), such that a substantial fraction of the driver cDNA and any tester cDNA hybridized thereto is selectively removed from the reaction mixture. The enriched (subtracted) tester cDNA pool may be subjected to one or more additional rounds of subtractive hybridization with a pool of labeled driver cDNA..." (Col. 22, lines 40-70).

Austin et al does not teach partitioning the RNA molecules before creating cDNA, or what portion of the RNA molecule the cDNA portion is derived from, or hybridization

of a probe nucleic acid sequence to the population of nucleic acids, or that the comparing of sequences is by determining the nucleotide sequence, or equalizing the representation of the molecules.

It would have been obvious to one of ordinary skill in the art at the time of the invention that the RNA molecules of interest may have been partitioned prior to cDNA copies being made from it. This would have been obvious because it would have been desirable to separate, or partition, the RNA population from other cellular material, or to partition certain RNA populations from others by using restriction enzymes or hybridization techniques. This would have allowed a certain portion of the cellular RNA to be characterized without the interference of undesired RNA transcripts, or other cellular materials. It would also have been obvious that the cDNA population may have been derived from any portion of the RNA that was desired, whether that be the 5' end, the 3' end, or an interior portion. This is because it would have been desirable to use any of those segments, depending on what the desired portion of the RNA was, and it was well known in the art that copies of any of those portions of RNA were easily copied into cDNA molecules. Also, it would have been obvious to hybridize a probe to the population of nucleic acids. This would have been obvious because it would have been another way of identifying which molecules of the target nucleic acids hybridized with molecules of the reference nucleic acid, instead of using ligands as taught by Austin. Probes would have been obvious because it was well known that they were easily detectable by fluorescent detection methods, and hybridizable to an array, etc. It would have also been obvious to use the method of Austin et al. to equalize the representation

of nucleic acids in a population. This is because Austin taught that two populations were hybridized together, and this would have equalized the representation of the populations by having only sequences which hybridized remain. This would have been obvious because it would have been advantageous to have two equalized population when comparing sequences, for instance.

5. Claims 9-17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Austin et al as applied to the claims above, and further in view of Sytkowski et al. (USPN 6,177,244).

As disclosed above, Austin et al. teaches "RNA prepared by conventional methods from a first cell population and RNA from a second cell population are separately reverse-transcribed and second-strand synthesized to form two pools of double-stranded cDNA a tester pool comprising sequences of the mRNA species desired to be enriched for, and a driver pool comprising the sequences desired to be subtracted from the tester pool. The two pools may be fragmented by endonuclease digestion (restriction endonuclease or non-specific endonuclease) if desired to enhance hybridization efficiency. The driver pool and tester pool are denatured and mixed together in a reaction mixture under hybridization conditions and incubated for a suitable hybridization period. The reaction mixture is contacted with a ligand which binds the recoverable label on the driver cDNA and which can be readily recovered from the reaction mixture (e.g., avidin attached to magnetic beads), such that a substantial fraction of the driver cDNA and any tester cDNA hybridized thereto is selectively removed from the reaction mixture. The enriched (subtracted) tester cDNA pool may be

subjected to one or more additional rounds of subtractive hybridization with a pool of labeled driver cDNA..." (Col. 22, lines 40-70).

Austin et al. does not teach ligating adapter oligonucleotides to the termini of the digested cDNA molecules, amplifying those products, separating the amplified products using gel electrophoresis, comparing the sizes of the populations, recovering the size-separated products and reamplifying them, or inserting the ligated adapter oligonucleotide into a cloning vector to form a vector-insert; transforming the vector-insert into a suitable host; culturing transformed host under conditions allowing for replication of the vector-insert; recovering the vector-insert from said host; and digesting the vector-insert with one or more restriction enzymes, thereby releasing said insert; and comparing the size of the insert to sizes of fragments generated by the same restriction enzyme or enzymes in said reference nucleic acid.

Sytkowski et al teaches a method used to isolate genes expressed differentially between two cell types or between cells treated in two different ways, or for isolation of differences between genomic DNA sequences. In a process of subtractive hybridization, Sytkowski et al. teaches the use of ligating linker, or adapter, oligonucleotides to the ends of the cDNA molecules. Sytkowski also teaches multiple rounds of subtractive hybridization, amplification, and gel electrophoresis. (Col. 48, line 4 through Col. 49, line 60). Sytkowski et al. also teaches the formation of a subtractive library using a cloning vector, and comparing the size fragments (Col. 50, lines 14-20).

It would have been obvious to one of ordinary skill in the art at the time of the invention to combine the teachings of Austin and Sytkowski. This is because it would

have been advantageous to use an adapter which was ligated to the end of the cDNA in order to amplify and identify the desired sequences. Furthermore, it would have been obvious to one of ordinary skill in the art at the time of the invention to use a gel to compare sizes of fragments. This would have allowed one of ordinary skill in the art to distinguish between fragments of different samples. Also, it would have been obvious to repeat those steps of amplification and subtractive hybridization in order to obtain the highest percentage of novel nucleic acids. It would have also been obvious to use a vector in order to create a library that would have been useful at a later date for comparison.

Summary

Claims 1, 19, and 27 are rejected under 35 U.S.C. 102(e). Claims 3-7, 9-18, and 20 are rejected under 35 U.S.C. 103(a).

No claims are allowable.

Response to Arguments

3. Applicant's arguments filed August 14, 2002 have been fully considered but they are not persuasive. In regards to the 35 U.S.C. 102(e) rejection, Applicant argues that "Applicant's invention generates collections of long or short fragments depending on the specifically partitioned cDNA molecules to provide a diversity of nucleic acid sequences". However, this is not the *claimed* invention, as Applicant has not set forth the length of the fragments in the claims. Applicant has also argued that "Austin fails to teach one of ordinary skill in the art to partition a cDNA population into one or more subpopulations..." However, as disclosed above, Austin teaches two pools of cDNA,

which are exposed to a restriction endonuclease. Applicant also argues that Austin fails to teach "sequenc[ing] a first nucleic acid sequence in the subpopulation to establish novelty of the sequence". However, Austin teaches "cDNA species remaining in the tester pool can be cloned, amplified, and/or sequenced for unambiguous sequence identification, such as the form of an EST sequence." Austin therefore teaches sequencing, and since Austin discloses that the sequence may be an EST, which by its nature is novel, as it is unknown, Austin fully anticipates the claims. Applicant also argues that "Austin fails to teach the skilled artisan to solve the problem of identifying novel sequences". Again, Austin teaches sequencing a cDNA population, which would have identified novel sequences.

4. Since Applicants arguments regarding the 35 U.S.C. 103(a) rejection rely on the arguments made above regarding Austin, they are not separately addressed. Applicant stated, however, on page 13 of the response that "Austin and Sytkowski references take mutually exclusive paths and reach different solutions to a similar problem." Applicant does not further expound, however, on what these mutually exclusive paths are, so they are not addressed herein.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Janell Taylor Cleveland, whose telephone number is (703) 305-0273.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached at (703) 308-1152.

Any inquiries of a general nature relating to this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Papers related to this application may be submitted by facsimile transmission. Papers should be faxed to Group 1634 via the PTO Fax Center using (703) 305-3014 or 305-4227. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG (November 15, 1989.)

Janell Taylor Cleveland

September 17, 2002

W. Gary Jones
Supervisory Patent Examiner
Technology Center 1600

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